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Sectoring patterns of spontaneous and radiation-induced somatic pink mutations in the stamen hairs of a temperature-sensitive mutable clone of *Tradescantia*

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ABSTRACT

The sectoring patterns of somatic pink mutations were analyzed in the stamen hairs of *Tradescantia* clone KU 20, a temperature-sensitive mutable clone. This clone is a blue/pink heterozygote, and its spontaneous pink mutation frequency increases up to about 40-fold at lower temperature. In order to elucidate the mutable nature of this clone, the sectoring patterns were analyzed on 1,123 spontaneous pink mutant events and on 2,725 pink mutant events induced by 0.606 and 1.28 Gy of gamma rays. The average number of pink cells per terminal pink mutant event (a row of pink cells including the terminal cell of a hair) occurred spontaneously was 7.40, whereas the number for the terminal pink mutant event induced by gamma rays varied from 3.33 to 9.88 depending on the post-irradiation days, i.e., increased gradually as the number of days proceeded, then was stabilized at the level of spontaneous mutations after about three weeks. The average number of pink cells per interstitial pink mutant event (a single pink cell or two or more contiguous pink cells between blue cells) was 1.97 for spontaneous mutations, while the number for induced mutations varied also depending on the post-irradiation days. The ratio of the number of interstitial pink mutant events against that of terminal pink mutant events was 1.35 for spontaneous mutations, but the ratio for induced mutations varied also with post-irradiation period reaching 2.89 at the peak, indicating that more interstitial pink mutant events are induced by gamma rays than terminal pink mutant events, as compared with spontaneous mutations. The frequency of multiple pink mutant sectors in a hair was more than four times higher than that expected from independent occurrences in case of spontaneous mutations, while the frequency was close to the expectation in induced mutations, suggesting that somatic recombination is involved as one of the major causes of spontaneous mutations in this mutable clone.

1. INTRODUCTION

Clone KU 20 was originally found by the author, among many *Tradescantia* clones collected, as one of those proven to be heterozygous for flower color (blue/pink), and was soon confirmed to be highly mutable spontaneously especially at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa, 1984). This clone

has been reported to exhibit as much as about 23- (Takahashi and Ichikawa, 1976) to 40-fold (Imai et al., 1991) differences in spontaneous somatic pink mutation frequency in the stamen hairs in the temperature range of 17.5 to 28.3°C, the mutation frequency being higher at lower temperature. This clone has therefore been regarded as a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992; Imai et al., 1991).

Diversely different spontaneous mutabilities have been reported among many different *Tradescantia* clones heterozygous for flower color (Sparrow and Sparrow, 1976; Takahashi and Ichikawa, 1976; Ichikawa, 1984), and, besides clone KU 20, at least five other highly mutable clones, BNL 0106 (Sparrow and Sparrow, 1976; Nauman et al., 1978), KU 13, KU 14, KU 17 and KU 24 (Ichikawa, 1984) have been described. Among these highly mutable clones, KU 20 is most mutable, being followed by clones BNL 0106 and KU 17 (Ichikawa, 1984).

On the other hand, the mutational responses of clone KU 20 to ionizing radiation have been reported to be rather similar to those of non-mutable or stable clones (Ichikawa and Takahashi, 1977; Ichikawa et al., 1991). The gamma-ray-induced somatic pink mutation frequency in this clone determined recently was essentially identical with those in stable clones BNL 02 and KU 9, when the spontaneous mutation frequency in clone KU 20 was relatively low (Ichikawa et al., 1991). The induced mutation frequency in clone KU 20 became significantly higher than those in these stable clones, only when the spontaneous mutation frequency in clone KU 20 was very high (Ichikawa et al., 1991). The extent of increase in the radiation-induced mutation frequency in clone KU 20 was nevertheless very much less than the increase in the spontaneous mutation frequency, suggesting different mechanisms of initiation and repair of spontaneous and radiation-induced mutations (Ichikawa et al., 1991).

The present study was performed to elucidate the mutable nature of clone KU 20, by analyzing the sectoring patterns of spontaneous and gamma-ray-induced pink mutations in the stamen hairs, especially those of multiple pink mutant sectors occurring in the same hairs.

2. MATERIALS AND METHODS

Materials used

Clone KU 20 is a triploid clone ($3x=18$) heterozygous for flower color (blue/pink; the blue color being dominant) and is highly mutable spontaneously especially at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa, 1984, 1992; Imai et al., 1991). The origin of this clone remains unknown, but it possesses part of the characteristics of *T. ohiensis* Raf., thus appears to be a hybrid involving this species (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992; Sanda-Kamigawara and Ichikawa, 1993).

The normal blue and mutant pink flower-color pigments of this clone have been

studied microspectrophotometrically, together with those of three stable clones, BNL 02, KU 27 and KU 9, and both the blue and pink pigments of clone KU 20 have been confirmed to be identical with those of any of the three stable clones (Sanda-Kamigawara and Ichikawa, 1993).

In the present study, potted plants of clone KU 20 bearing young inflorescences just before initiating blooming were selected as materials from those which had been grown either in a growth chamber (Sherer, CEL 38–15) or in the greenhouse. The environmental conditions in the growth chamber were 23 ± 0.5°C during the day and 20 ± 0.5°C at night, 60% humidity, and 16-hr day length with the maximum light intensity of 23 klx from Sylvania VHO cool white fluorescent tubes (22 klx) and incandescent bulbs (1 klx) for 12 hr. On the other hand, the temperature and the humidity in the greenhouse, in which a 16-hr day length had been maintained, had been continuously recorded with a self-temperature/humidity recorder, and the maximum and the minimum temperatures recorded during the preceding two weeks were 37.4 and 15.2°C, respectively, averaging 22.8°C.

Two potted plants (one from the growth chamber and the other from the greenhouse) were employed for studying spontaneous mutations, and three potted plants (two from the growth chamber and one from the greenhouse) were used for analyzing gamma-ray-induced mutations. After carrying them to and back from the gamma field for gamma-ray treatments as described below, they were continuously grown in the growth chamber throughout the experimental period.

Gamma-ray treatments

Treatments with ^{60}Co gamma rays were carried out in the gamma field of the Institute of Radiation Breeding, National Institute of Agricultural Science, Ohmiya, Ibaraki. The five potted plants were carried to the institute on the day when they were selected, and three of them were exposed to gamma rays for 20 hr starting at noon of the next day. The two plants used for studying spontaneous mutations were placed in the control field of the institute during the gamma-ray treatments. After the gamma-ray treatments, they were carried back to our laboratory. The exposure data were obtained simultaneously with thermoluminescence dosimeter (TLD) elements (National, UD-170L) attached to individual potted plants (two TLD elements per plant), and with a thermoluminescence reader (National, UD-502B). The exposure data obtained in R were then converted into absorbed doses in Gy with a converting factor of 9.57×10^{-3} (i.e., 1 R=9.57 mGy). The doses applied were 0.606 Gy for one plant from the growth chamber and 1.28 Gy for one plant each from the growth chamber and the greenhouse.

The temperature during the gamma-ray treatments was also recorded, and was found to have changed between 15.3 and 25.6°C, averaging 19.0°C. The temperature range during transporting the potted plants to and from the gamma field was measured with a maximum/minimum thermometer, and was found to have

remained between 22.6 and 25.3°C.

Scoring of mutations

The methods used for scoring pink mutations in the stamen hairs in the present study were mostly identical to those described elsewhere recently (Ichikawa et al., 1990, 1991, 1993; Ichikawa and Ishii, 1991a, b; Sanda-Kamigawara et al., 1991; Ichikawa, 1992; Shima and Ichikawa, 1994). Briefly, all the flowers that opened during the scoring period of post-irradiation days 2 to 25 were collected daily (for 24 days starting from the fourth day after selecting the materials); the numbers of stamen hairs and of pink mutant events were scored on each of six stamens, recording the sectoring pattern of every pink mutant event found; and the number of hair cells was also counted on ten representative hairs each of two oppositely located stamens per flower as described earlier (Ichikawa and Takahashi, 1977, 1978; Ichikawa and Ishii, 1991b), to estimate the average number of cells per hair for calculating mutation frequency per hair-cell division (Ichikawa and Takahashi, 1977, 1978; Ichikawa, 1984, 1992; Ichikawa et al., 1990, 1991, 1993; Ichikawa and Ishii, 1991a, b; Sanda-Kamigawara et al., 1991). A pink mutant event has been defined to represent the result of a single mutation, being principally a single pink cell or two or more contiguous pink cells in a hair, and the further details of the definition of the pink mutant events have been described elsewhere (Ichikawa, 1981a, 1992; Sanda-Kamigawara et al., 1991; Ichikawa et al., 1993). The sectoring patterns of multiple pink mutant events in the same hairs were especially carefully recorded in the present study.

3. RESULTS AND DISCUSSION

Spontaneous mutation frequency

The spontaneous pink mutation frequency in the stamen hairs of the control plant selected from those which had been grown in the growth chamber was 18.7 ± 1.1 pink mutant events per 10^3 hairs, pooling the data for the entire scoring period. On the other hand, the spontaneous mutation frequency in the control plant selected from those which had been grown in the greenhouse was 28.5 ± 1.0 pink mutant events per 10^3 hairs, also pooling the data for the same period. Since there was a difference in the average numbers of cells per hair between the two control plants (23.24 and 25.02 for the plants from the growth chamber and the greenhouse, respectively), which was not negligible, it is considered to be more appropriate to compare the spontaneous mutation frequencies per hair-cell division. The values obtained were 8.39 ± 0.48 and 11.9 ± 0.4 pink mutant events per 10^4 hair-cell divisions, respectively, for these two plants. The value per 10^4 hair-cell divisions for the plant from the greenhouse is about 40% higher than that for the plant continuously grown in the growth chamber, but the higher pooled value reflected higher mutation frequencies during earlier 11 scoring days after

moving the plant from the greenhouse to the growth chamber. Namely, the numbers of pink mutant events per 10^4 hair-cell divisions in this plant for earlier 11 and later 13 days were 14.9 ± 0.6 and 8.53 ± 0.51, respectively, the latter being almost identical with the mutation frequency in the plant which had been continuously grown in the growth chamber. The higher spontaneous mutation frequency in the earlier 11 days is considered to be the results influenced by the much fluctuated temperature in the greenhouse, as observed earlier (Takahashi and Ichikawa, 1976; Ichikawa, 1984; Imai et al., 1991). The pattern of decrease in spontaneous mutation frequency observed after moving the plant from the greenhouse to the growth chamber agrees with our earlier report (Imai et al., 1991), but the mutation frequency observed in the plant continuously grown in the growth chamber was about two times higher than that in the earlier study (Imai et al., 1991).

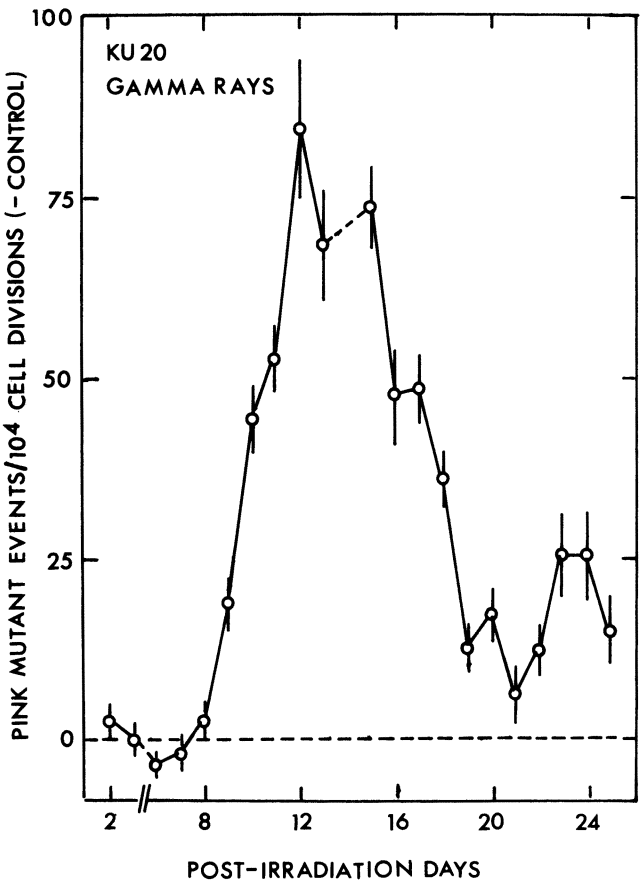

Fig. 1. The daily change of the number of pink mutant events per 10^4 hair-cell divisions observed in the plant (from the greenhouse) exposed to 1.28 Gy of ^{60}Co gamma rays.

Gamma-ray-induced mutation frequency

The pink mutation frequency in the stamen hairs observed in the plants exposed to gamma rays started to increase on post-irradiation day 9, and reached its peak on post-irradiation day 12 or 13. The daily change of the mutation frequency per 10^4 hair-cell divisions observed in the plant (from the greenhouse) exposed to 1.28 Gy of gamma rays is shown in Fig. 1, as an example. It is seen in this figure that considerably high mutation frequencies lasted longer than three weeks.

The gamma-ray-induced mutation frequencies pooled for the 7-day peak period (post-irradiation days 11 to 17) in this plant was 63.5 ± 2.7 pink mutant events per 10^4 hair-cell divisions (the control frequency was subtracted). This value fits to one of the two regression lines obtained earlier (Ichikawa et al., 1991), that is, the regression line obtained when the spontaneous mutation frequency in this clone was very high.

Sectoring patterns of terminal pink mutant events

In the present study, 1,123 pink mutant events occurred spontaneously and 2,725 pink mutant events induced by gamma rays were observed in total. In this classification, all the pink mutant events observed in the two control plants and those observed during post-irradiation days 2 through 8 in the three plants irradiated were regarded as those occurred spontaneously (since radiation effect first appeared on post-irradiation day 9), while all the pink mutant events observed in the latters after post-irradiation day 9 were regarded as those induced by gamma rays.

Among these pink mutant events, 423 and 764 were single terminal pink mutant events occurred spontaneously and induced by gamma rays, respectively. A single terminal pink mutant event means a single pink terminal cell or a row of pink cells including the terminal cell of a hair, without any other pink cells in the hair, as shown in Fig. 2 (the second and third at the left). The distribution patterns of the number of pink cells per single terminal pink mutant event were studied separately for spontaneous and gamma-ray-induced mutant events, and, in case of the latters, also separately for post-irradiation periods. The results obtained are presented in Table 1.

The number of pink cells per single terminal pink mutant event occurred spontaneously averaged 7.40, although the number showed a wide variation from 1 up to 25 (Table 1). On the other hand, the average number of pink cells per single terminal pink mutant event induced by gamma rays varied from 3.33 to 9.88 depending on the post-irradiation days, i.e., increased gradually as the number of days proceeded, then was stabilized at the level of spontaneous mutations after about three weeks (Table 1). The variation in the number of pink cells also showed a similar tendency. These changes in the number of pink cells per single terminal pink mutant event after irradiation agree well with earlier reports on

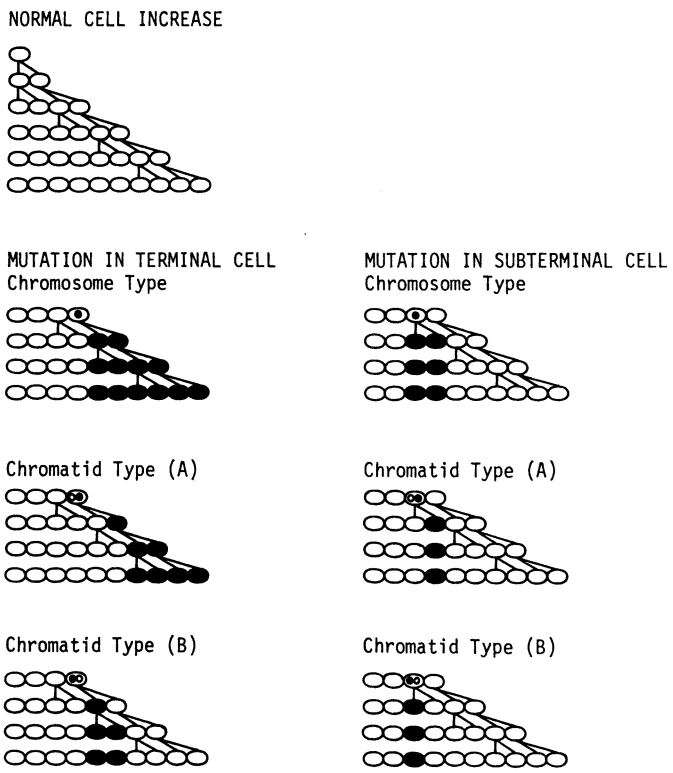

Fig. 2. Diagrams to illustrate the pattern of cell increase and the appearance of mutations in the stamen hairs of *Tradescantia*. Interstitial cells also divide but only occationally (Ichikawa et al., 1969), except for early stage of hair growth (Mericle and Hazard, 1980). Terminal pink mutant events are resulted from chromosome- and chromatid-type mutations occurred in the terminal cell, while interstitial pink mutant events arise from chromosome- and chromatid-type mutations in the subterminal cell or from chromatid-type mutations in the terminal cells. This figure is a part of Fig. 7.4 in Ichikawa (1981b) and of Fig. 2 in Ichikawa (1992).

two stable clones, BNL 2031 (Ichikawa and Sparrow, 1968) and BNL 02 (Ichikawa et al., 1969).

Sectoring patterns of interstitial pink mutant events

The numbers of single interstitial pink mutant events observed as those occurred spontaneously and induced by gamma rays were 570 and 1,371, respectively. A single interstitial pink mutant events means a single pink cell or two or more contiguous pink cells between blue cells in a hair, without any other pink cells in the hair, as shown in Fig. 2 (the bottom at the left and three at the right). The distribution patterns of the number of pink cells per single interstitial pink mutant event were studied, as done for single terminal pink mutant events, and the results obtained are shown in Table 2.

Table 1. Changes of the distribution pattern of the number of cells per single terminal pink mutant event after gamma-ray treatments

No. of cells/ mutant event	Sponta-neous mutations* (%)	Induced mutations on post-irradiation days						
		9, 10 (%)	11, 12 (%)	13, 14 (%)	15, 16 (%)	17, 18 (%)	19–21 (%)	22–25 (%)
1	11.1	43.6	26.6	12.7	7.7	6.7	10.8	10.3
2	12.5	18.2	27.3	25.5	7.1	10.4	10.0	11.8
3	6.4	12.7	11.7	11.8	5.8	4.4	4.2	2.9
4	7.6	3.6	6.3	12.7	10.9	7.4	5.8	10.3
5	6.4	1.8	4.7	4.9	8.3	1.5	6.7	5.9
6	7.3	5.5	3.9	7.8	6.4	2.2	5.8	8.8
7	5.9	1.8	3.9	5.9	3.2	4.4	9.2	1.5
8	4.3	1.8	0.8	3.9	7.1	5.9	7.5	7.4
9	5.4	1.8	2.3	2.9	7.1	3.0	7.5	2.9
10	4.3	1.8	5.5	2.9	4.5	5.9	4.2	5.9
11	6.4	1.8	0.8	2.9	6.4	5.2	3.3	4.4
12	4.5	1.8	1.6	2.0	5.1	7.4	3.3	7.4
13	4.5		2.3	2.0	6.4	8.9	1.7	4.4
14	2.8		1.6	1.0	3.8	2.2	4.2	2.9
15	1.7	3.6	0.8		3.8	5.2	1.7	4.4
16	2.6			1.0	1.9	3.0	2.5	
17	2.8				3.2	3.7	4.2	1.5
18	0.2				0.6	3.0	1.7	
19	0.9					3.7	3.3	2.9
20	0.5					2.2	1.7	
21	0.7				0.6	0.7		
22	0.5					1.5		
23						0.7		
24	0.2							1.5
25	0.5					0.7		
26								2.9
27								
28							0.8	
Total no.	423	55	128	102	156	135	120	68
Average no. of cells /event	7.40	3.33	3.89	4.61	7.87	9.88	8.11	8.15

* Single terminal pink mutant events observed in control plants plus those observed in irradiated plants on post-irradiation days 2–8.

The number of pink cells per single interstitial pink mutant event occurred spontaneously averaged 1.97 (Table 2), showing a much smaller variation than that per single terminal pink mutant event (see Table 1). On the other hand, the number of pink cells per single interstitial pink mutant event induced by gamma rays varied from 1.39 to 2.33 depending on the post-irradiation days (Table 2), exhibiting also a much smaller variation than that per single terminal pink mutant

Table 2. Changes of the distribution pattern of the number of cells per single interstitial pink mutant event after gamma-ray treatments

No. of cells/ mutant event	Sponta-neous mutations* (%)	Induced mutations on post-irradiation days						
		9, 10 (%)	11, 12 (%)	13, 14 (%)	15, 16 (%)	17, 18 (%)	19–21 (%)	22–25 (%)
1	39.1	64.8	65.6	63.3	53.0	39.0	48.6	51.0
2	42.3	31.4	26.6	32.7	32.5	30.0	27.4	33.8
3	10.2	3.8	4.7	3.5	7.5	14.0	7.5	6.2
4	4.4		1.6	0.5	3.5	8.0	4.2	3.4
5	1.9		0.8		1.5	3.0	4.7	2.8
6	1.4				1.5	2.5	3.3	0.7
7	0.2		0.4			1.5	0.9	
8			0.4			0.5		
9					0.5	1.0	0.9	
10	0.4						0.9	1.4
11								
12	0.2					0.5		
13								0.7
14							0.9	
15							0.5	
Total no.	570	159	256	199	200	200	212	145
Average no. of cells /event	1.97	1.39	1.49	1.41	1.76	2.33	2.31	1.92

* Single interstitial pink mutant events observed in control plants plus those observed in irradiated plants on post-irradiation days 2–8.

event (see Table 1).

It is clear in Table 2, however, that the proportions of single-cell interstitial pink mutant events are mostly obviously higher than two-cell interstitial pink mutant events in gamma-ray-induced mutations, as compared with the case of spontaneously occurred mutations. This seems to indicate that chromatid-type mutations were more frequently induced in the subterminal cells by gamma rays than chromosome-type mutations (see Fig. 2), although the possibility of involving the loss of reproductive integrity (Ichikawa and Sparrow, 1968; Ichikawa et al., 1969, 1978; Ichikawa, 1972) occurred in the subterminal cells can not be excluded.

The ratio of interstitial against terminal pink mutant events

The ratio of the number of single interstitial pink mutant events against that of single terminal pink mutant events was also investigated, since the former events are mostly the results of both chromosome- and chromatid-type mutations occurred in the subterminal cells (partly resulted from 50% of chromatid-type mutations in the terminal cells) of young stamen hairs, while the latter events are resulted

Table 3. Frequencies of single terminal, single interstitial, whole-hair, and multiple pink mutant events after gamma-ray treatments

Post-irradiation days	Single terminal (%)	Single interstitial (%)	Whole hair (%)	Multiple (%)	Interstitial /Terminal
Control+2–8	423 (38.5)	570 (51.8)	20 (1.8)	87 (7.9)	1.35
9, 10	55 (24.4)	159 (70.7)	0 (0.0)	11 (4.9)	2.89
11, 12	128 (30.5)	256 (61.1)	0 (0.0)	35 (8.4)	2.00
13, 14	102 (31.0)	199 (60.5)	0 (0.0)	28 (8.5)	1.95
15, 16	156 (39.0)	200 (50.0)	6 (1.5)	38 (9.5)	1.28
17, 18	135 (30.0)	200 (44.4)	54 (12.0)	61 (13.6)	1.48
19–21	120 (22.7)	212 (40.1)	132 (25.0)	65 (12.3)	1.77
22–25	68 (19.4)	145 (41.4)	94 (26.9)	43 (12.3)	2.13
Total	764 (28.3)	1,371 (50.7)	286 (10.6)	281 (10.4)	1.79

solely from mutations (all of chromosome type and 50% of chromatid type) in the terminal cells of young stamen hairs (see Fig. 2). The results obtained are presented in Table 3.

The ratio for spontaneous mutations was 1.35 (Table 3), and this value means that 35% more single interstitial mutant events occurred spontaneously than single terminal mutant events. This ratio must be reflecting the nature of cell increase in *Tradescantia* stamen hairs (Ichikawa et al., 1969; Ichikawa, 1981b, 1992).

The ratio for gamma-ray-induced mutations, on the other hand, varied depending on post-irradiation days, being higher on earlier post-irradiation days (2.89 on post-irradiation days 9 and 10 was the highest value) as seen in Table 3. It indicates that, as compared with the case of spontaneous mutations, much more mutations are induced in the subterminal cells by gamma rays than in the terminal cells, and/or that much more chromatid-type mutations than chromosome-type ones are induced in the terminal and subterminal cells by gamma rays. If the former is correct, this finding may suggest differential radiosensitivities and/or repairing abilities between the terminal and subterminal cells. Considering the high frequencies of single-cell interstitial pink mutant events mentioned above (see Table 2), it seems most likely that more chromatid-type mutations are induced in the subterminal cells by gamma rays, since such single-cell interstitial pink mutant events are the results of chromatid-type mutations occurred in the subterminal cells (see Fig. 2). It should be noted, however, that the loss of reproductive integrity caused by gamma rays (Ichikawa and Sparrow, 1968; Ichikawa et al., 1969, 1978; Ichikawa, 1972) still remains as another possible cause of these results.

Besides these single terminal and interstitial pink mutant events, entirely pink hairs without any blue cells were observed. While 20 such entirely pink hairs

occurred spontaneously were observed evenly throughout the scoring period, those induced by gamma rays amounted to as many as 286, and none of them appeared before post-irradiation days 15 and 16 (Table 3). The appearance of such entirely pink hairs on later post-irradiation days reflects that stamen hairs initiate to grow on the stamen filament 15 (at the distal part) to 18 days (at the basal part) before flowering (Ichikawa et al., 1969; Mericle and Hazard, 1980; Ichikawa, 1992).

Analysis of multiple pink mutant sectors

Many hairs were found to have multiple pink mutant sectors. Namely, such multiple pink mutant sectors occurred in the same hairs were found in 368 hairs in total, and 87 and 281 of them had spontaneous and gamma-ray-induced multiple pink mutant sectors, respectively (Table 3).

In order to analyze these multiple pink mutant sectors, they were classified first into three categories of double, triple and quadruple pink mutant sectors, then by whether the multiple sectors in each hair are separated by a single blue cell or by two or more blue cells. The results of the classification are presented in Table 4. Double pink mutant sectors occupied 90.8% of all multiple sectors, and triple and quadruple pink mutant sectors were 7.6 and 1.6%, respectively.

Multiple pink mutant sectors each of which was separated by a single blue cell occupied 55.2%, and those separated by two or more blue cells were 42.1%. The remaining 2.7% were those in which at least two out of three or four pink mutant sectors were separated by two or more blue cells. It should be noted, however, that the frequencies of multiple pink mutant sectors separated by one blue cell and by two or more blue cells were 65.5 and 33.3%, respectively, for spontaneous mutations, while they were 51.9 and 44.8%, respectively, for induced mutations.

Table 4. Frequencies of various types of multiple pink mutant events

Post irradiation days	Separated by						Others*	
	One blue cell			Two or more blue cells				
	Double	Triple	Quadruple	Double	Triple	Quadruple	Triple	Quadruple
Control+2–8	56	1		27	2		1	
9, 10	7			4				
11, 12	19			15			1	
13, 14	12	1		14			1	
15, 16	16	1	1	19				1
17, 18	28	4		23	3	1	2	
19–21	30	1	1	27	3	1	2	
22–25	23	2		14	2		1	1
Total	135	9	2	116	8	2	7	2

* Separated partly by one blue cell and partly by two or more blue cells.

That is, the proportion of multiple pink mutant sectors each of which was separated by a single blue cell was obviously higher in spontaneous mutations (see Table 4).

The numbers of single, double, triple and quadruple pink mutant sectors observed are listed in Table 5, pooling the data for whole scoring period for those occurred spontaneously, and separately for seven post-irradiation periods for those induced by gamma rays. The expected numbers of double, triple and quadruple pink mutant sectors based on simultaneous and independent occurrences of two to four sectors (calculated based on the frequencies of single pink mutant events) are also shown in this table.

Table 5. Expected and observed numbers of multiple pink mutant events

Post-irradiation days	No. of hairs observed	No. of single mutant events	No. of single mutant events /10^4 cell div.* (±SE)	Double		Triple		Quadruple	
				Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Control+2–8	45,091	993	9.42±0.30	22	83 (27)**	0.48	4 (3)	0.011	0
9, 10	2,818	214	36.5 ±2.5	16	11 (4)	1.2	0	0.094	0
11, 12	4,362	384	41.7 ±2.1	34	34 (15)	3.0	1 (1)	0.26	0
13, 14	2,636	301	56.2 ±3.2	34	26 (14)	3.9	2 (1)	0.45	0
15, 16	3,680	356	49.0 ±2.6	34	35 (19)	3.3	1 (0)	0.32	2 (1)
17, 18	5,109	335	31.4 ±1.7	22	51 (23)	1.4	9 (5)	0.094	1 (1)
19–21	9,145	332	16.2 ±0.9	12	57 (27)	0.44	6 (5)	0.016	2 (1)
22–25	5,727	213	17.2 ±1.2	7.9	37 (14)	0.29	5 (3)	0.011	1 (1)

* Average cell numbers per hair used for calculating the mutation frequencies per 10^4 hair-cell divisions were as follows:

Control+2–8: 24.37 Days 9, 10: 21.83 Days 11, 12: 22.13 Days 13, 14: 21.31
Days 15, 16: 20.74 Days 17, 18: 21.87 Days 19–21: 23.45 Days 22–25: 22.57

** Figures in parentheses are those of multiple pink mutant events separated by two or more blue cells at least in part.

It is seen in this table that the observed number of double pink mutant sectors occurred spontaneously was nearly 4 times larger than the expected number, and the number of triple pink mutant sectors observed as those occurred spontaneously was about 8 times more than expected. On the other hand, the observed numbers of double pink mutant sectors induced by gamma rays were mostly close to the expected numbers, and the numbers of triple and quadruple pink mutant sectors observed were even smaller than expected, until post-irradiation day proceeded to day 16 (Table 5), differing greatly from the case of spontaneous mutation. In later post-irradiation periods, multiple pink mutant sectors were much more frequently observed than expected, but this is considered to be mainly due to increasing proportions of spontaneously occurring multiple pink mutant sectors in those later periods, because the induced mutation frequencies in these

periods decrease considerably (see Fig. 1). The higher frequencies of multiple pink mutant sectors in later post-irradiation periods are also considered to be related to the participation of interstitial cells in hair growth (cell proliferation) at earlier stage of hair growth (Mericle and Hazard, 1980).

Such a clear difference in the occurrences of multiple pink mutant sectors between spontaneous and gamma-ray-induced mutations suggests that somatic recombinations, which produce pairs of homozygous blue and homozygous pink daughter cells from blue/pink heterozygous cells, are also involved in producing a significant part of spontaneous mutations in this mutable clone, and that the occurrences of two somatic recombinations or of one somatic recombination plus one somatic mutation in a hair are the causes of the spontaneously occurring multiple pink mutant sectors.

The possibility of involvement of somatic recombinations in producing multiple pink mutant sectors in the stamen hairs of *Tradescantia* clone BNL 02 (non-mutable) was suggested earlier (Mericle and Mericle, 1967, 1973), and the occurrence of somatic recombinations was confirmed in the stamen hairs of a purple-flowered clone of *T. hirsuticaulis* Small (BNL 2091, also non-mutable) that produces blue/red twin spots when a somatic recombination occurs (Christianson, 1975). The latter study analyzed both spontaneous and gamma-ray-induced mutations, and concluded that the predominant cause of spontaneous twin spots and an important cause of induced twin spots were somatic recombinations. Similar conclusion was also reported earlier in *Glycine max* (Vig, 1973). Therefore, it is conclusive that somatic recombination plays an important role in producing multiple mutant sectors in *Tradescantia* stamen hairs.

The present finding of a clear difference in the occurrences of multiple pink mutant sectors between control and gamma-rayed plants is considered to be related to the mutable nature of clone KU 20 which is especially mutable spontaneously (Ichikawa and Takahashi, 1977; Ichikawa et al., 1991). Considering that this clone is especially highly mutable spontaneously at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992; Imai et al., 1991; Ichikawa et al., 1991), and that somatic recombination is considered to be one of the major causes of the spontaneous mutations in this clone as discussed above, it becomes more likely than previously thought (Imai et al., 1991) that a recombinational or postreplication repair, which has not yet been proved to be operative in higher plants, is involved in the mutable nature of this clone.

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